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Blood Groups and Urinary Micro-organisms*

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The evidence for selection in blood group polymorphism is limited (Reed, 1961). If infection is considered as a selective force it would be of interest to determine whether subjects of different blood types possess varying degrees of resistance to infectious diseases. Muschel and Osawa (1959) showed cross-reactivity between human blood groups substance B and *Esch. coli* 086, and suggested a possible influence of blood groups in resistance to infection against those microbial agents that may possess blood groups antigens. Eichner, Finn, and Krevans (1963) in a study on the relations between serum antibody levels and the ABO blood group polymorphism found that the mean antibody titre of anti-*coli* 086 B7 was lowest in group O and highest in group AB. They suggested that if selective forces influencing ABO polymorphism were active, individuals of certain blood groups might be at a disadvantage in combating any infection due to an organism carrying the cross-reacting antigen.

It is difficult to design an experiment for a clinical approach to this problem. At the clinical level, there are many ecological factors that affect the resistance of individuals to specific infections. Infectious diseases are complex phenotypic entities. An experiment analysing the association between a genetic character and a specific infectious disease must take these difficulties into account. The relationship between gene and infection needs to be studied directly in relation to the presence of the micro-organism rather than in relation to clinical infection. We think that it is possible to perform a preliminary analysis of the selective disadvantages of some given genotypes, investigating through a prospective monofactorial method (Li, 1961) the association of marker genes with the presence of the micro-organism affecting the urinary tract of human beings.

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Subjects and Methods

The material included all patients admitted in Division B of Medicine in the Hospital Jose Joaquin Joaquin Aguirre, Santiago, Chile, between October 1, 1963 and August 1, 1964 excluding severely ill patients. The age and sex distribution of our sample is shown in Table I.

TABLE I

DISTRIBUTION OF SAMPLE BY AGE AND SEX

Age	Males		Females		Total	
(yr.)	No.	%	No.	%	No.	%
10–19	31	7·3	33	9·6	64	8·3
20–29	58	13·6	65	18·9	123	16·0
30–39	72	16·9	59	17·2	131	17·0
40–49	87	20·5	59	17·2	146	19·0
50–59	89	20·9	68	19·5	187	20·4
60–69	54	12·7	36	10·5	90	11·7
70–79	28	6·6	20	5·8	48	6·2
80–89	6	1·4	4	1·2	10	1·3
	425	10·0	344	100·0	769	100·0

During the first three days in hospital the following studies were carried out.

(1) Urine specimens were collected in sterile tubes by the midstream method and incubated for bacteriological study within two hours. Cultures were read after 48 hours incubation at 37°C. The colonies, which appeared in cultures, were classified according to Bergey and Kauffman.

(2) The typing of blood groups ABO and MN was performed with fresh blood within 8 hours of sampling in the Blood Grouping Laboratory in the Instituto Bacteriologico de Chile, according to standard methods used there. Anti-sera anti-M and anti-N, of the Certified Blood Donor Service, Jamaica, N.Y., U.S.A., were used.

(3) Phenylthiocarbamide taste sensitivity was studied according to the method of Harris and Kalmus (1950), using 15 solutions. The sample was classified according to birthplace and educational status.

Gene frequencies of 'marker' genes were calculated according to Li (1961). The statistical significance of the difference between the distribution of a given genotype and a given micro-organism was tested by the chi square test (Yates correction) and its correlation coeffi-

TABLE II

GENE FREQUENCIES OF ALLELES OF ABO AND MN LOCI

	Number	Frequency
ABO System		
Phenotypes		
Group A	227	0·310
Group B	81	0·110
Group AB	19	0·026
Group O	404	0·552
	731	
Alleles		
(p) I_A		0·1856 ± 0·0105
(q) I_B		0·0710 ± 0·0067
(r) I_O		0·7434 ± 0·0065
χ^2 (Taylor and Prior) = 0·161; p > 0·20		
NM System		
Phenotypes		
Group M	244	0·373
Group MN	329	0·495
Group N	88	0·132
	661	
Alleles		
L_M		0·618 ± 0·013
L_N		0·382 ± 0·0.3
χ^2 (Fisher) = 1·94; p > 0·05		

cient, through a prospective approach (Li, 1961). The conditional probability of having a micro-organism "z", given that the individual carries a genetic character "y", was calculated according to the equation:

$$(1)\ P(z \mid y) = N(zy) \mid N(y),$$

where N (zy) represents the number of patients with micro-organism "z", the character "y", and N (y) the number of patients with genetic character "y". The relative risk or relative susceptibility for each genotype to have a given micro-organism was defined by the ratio between two conditional probabilities according to the equation:

$$(2)\ x = P(z_1 \mid y_1)\, P(z_2 \mid y_2)$$

where numbers 1 and 2 represent two different genotypes or set of genotypes.

Results

Table II shows the incidence of blood groups of the ABO and MN systems with the estimated gene frequencies of their alleles. The genotype distribution of both loci was in equilibrium according to the Hardy-Weimberg Law. The frequency of non-tasters (solution 4, PTC) was found to be 12·3%, a figure in good agreement with Chilean samples (Alvial and Henckel, 1944; Covarrubias, 1964; Cruz-Coke and Iglesias, 1963).

Table III shows the distribution of genetic characters in relation to birthplace. Two-thirds of the subjects had been born outside Santiago, showing the classical Chilean strong process of urban immigration. Only 3% of our sample were foreign-born subjects. European ancestry is marked by the high percentage of non-tasters (39·1%). The educational status of the patients was as follows: 10% were illiterate, 51% had primary schooling, and only 3% had had a university education. This composition represents a typical Chilean population of low socio-economic status.

Urine culture could only be done in 673 (88·1%) of the patients, because all those recently treated with antibiotics were excluded from examination. A positive culture was found in 465 patients (69%). The percentage of contamination was high and the coagulase-negative *Staphylococcus*

TABLE III

DISTRIBUTION OF LOCI ABO, MN, AND Tt IN RELATION TO BIRTHPLACE

Phenotypes	Chilean Regions								Foreign		Total	
	North		Central		South		Santiago					
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A	18	25·4	90	30·9	20	29·2	81	33·5	8	33·9	226	31·0
B	8	11·3	34	11·7	12	12·1	21	8·7	4	16·7	79	10·9
AB	3	4·2	6	2·1	0	0	9	3·7	1	4·2	19	2·6
O	42	59·1	161	55·3	58	58·7	131	54·1	11	45·8	403	55·4
	71		291		99		242		24		727	
M	25	39·7	102	38·5	33	36·3	77	35·6	9	40·9	246	37·4
MN	28	44·4	132	49·8	47	51·1	108	50·0	10	45·5	325	49·4
N	10	15·9	31	11·7	11	12·0	31	14·4	3	13·6	86	13·1
	63		265		91		216		22		657	
TT-Tt	64	83·1	263	88·6	100	89·3	222	90·6	14	60·9	653	87·7
tt	13	16·9	34	11·4	12	10·7	23	9·4	9	39·1	91	12·3
	77		297		112		245		23		744	

TABLE IV

DISTRIBUTION OF PHENOTYPES OF LOCI ABO, MN, AND Tt IN RELATION TO FIVE URINARY MICRO-ORGANISMS

Phenotypes	*Esch. coli*		*Staph. albus haemolyticus*		*Strep. faecalis*		*Proteus vulgaris*		*K pneumoniae*		Total Sample	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A	57	33·2	45	32·6	22	31·5	21	38·2	11	24·5	211	32·8
B	29	16·8	16	11·6	14	20·0	3	5·5	4	8·9	72	11·1
AB	4	2·3	5	3·5	3	4·3	2	3·6	2	4·4	14	2·2
O	82	47·6	72	52·2	31	44·2	29	52·7	28	62·2	342	53·9
	172		138		70		55		45		644	
M	52	33·3	46	36·6	21	31·8	17	31·4	16	40·0	216	35·8
MN	82	52·5	64	50·8	35	53·1	29	53·8	16	40·0	306	50·9
N	22	14·2	16	12·6	10	15·1	8	14·8	8	20·0	80	13·3
	156		126		66		54		40		602	
TT-Tt	153	86·4	122	86·5	56	75·6	51	89·4	39	86·6	581	88·1
tt	24	13·6	19	13·5	18	24·4	6	10·6	6	13·4	78	11·9
	177		141		74		57		45		659	

albus haemolyticus was present alone in 113 cases (17%). The most frequent bacteria found was *Esch. coli* (38% of the positive cultures and 26% of the total bacteriological sample).

The distribution of genetic characters in relation to the five most frequently encountered micro-organisms in urine culture is shown in Table IV. There is an excess of group B in relation to *Esch. coli* and *Streptococcus faecalis*, and an excess of non-tasters in relation to *Strep. faecalis*. The degree of association of B versus non-B blood groups with micro-organism is analysed in Table V. This association is significant at the 1% level only with *Esch. coli*. A higher degree of association is reached with B versus O blood groups and *Esch. coli* ($x = 1{\cdot}7$; $\chi^2 = 7{\cdot}65$; $r = 0{\cdot}13$). The association of non-taster individuals to *Strep. faecalis* is also highly significant with a relative susceptibility 139% higher than tasters ($x = 2{\cdot}39$; χ^2 11·15; r 0·13). No other significant association was found in this analysis.

Discussion

The gene frequencies of the ABO alleles of this sample are in good agreement with the large Chilean sample of Sandoval (1941). Our bacteriological investigations agree with the findings of De Wardener (1958) that only 25% of urine samples obtained from clinically 'normal' subjects were sterile. As a diagnostic procedure, urine culture is less satisfactory than microscopical examination, but our investigation was designed to establish the presence or absence of a given micro-organism in the urinary tract, without considering the number of colonies or other parameters of a 'clinical infection'.

Our investigation shows clearly that B subjects have a probability 50% higher than that of non-B

TABLE V

ASSOCIATION AND RELATIVE SUSCEPTIBILITY OF INDIVIDUALS OF B VERSUS NON-B BLOOD GROUPS WITH URINARY MICRO-ORGANISM IN A SAMPLE OF 644 (N) PATIENTS

Urinary Micro-organisms	N (z)	x*	χ^2†	p	r‡	p
Esch. coli	172	1·50	6·86	0·01	0·103	0·01
Staph. albus haemolyticus	138	1·05	0·01	0·30	0·005	0·20
Strep. faecalis	70	2.00	5·19	0·05	0·089	0·05
Proteus vulgaris	55	0·49	1·58	0·20	0·048	0·10
K. pneumoniae	45	0·83	0·12	0·20	0·013	0·10
K. pneumoniae	45	0·83	0·12	0·20	0·013	0·10

* x = relative susceptibility of B versus A, AB, and O individuals.
† χ^2 = with Yates correction.
‡ r is correlation coefficient $\sqrt{(\chi^2/N)}$.

subjects, and 70% higher than O subjects, of contracting urinary infection with *Esch. coli*. This relative susceptibility is higher than those described between ABO blood groups and duodenal ulcer and cancer of the stomach (Reed, 1961). To explain this association we can consider the hypothesis of Muschel and Osawa (1959), that agglutinins anti-B probably exert a bactericidal effect upon the strains of *Esch. coli* 086. We assume that B subjects are more predisposed to be infected with *Esch. coli* because, since they do not carry agglutins anti-B, they are unable to destroy coliform organisms. The association found between non-tasters and infection with *Strep. faecalis* is not easily explained, but our findings seem to support the hypothesis of Eichner *et al.* (1963) that selective forces which influence blood group polymorphism are probably active at the present time.

Summary

A sample of 727 patients admitted to hospital was 'marked' with three autosomic loci (ABO, MN, and Tt) and the urines were cultured to assess the association of genes with bacteria *in vivo*. Significant associations were discovered between individuals with B blood groups and *Esch. coli*, as also between non-tasters to phenylthiocarbamide and *Strep. faecalis*.

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